Cell Reports
Previews

Tumor Suppression by p53: Is Apoptosis Important or Not?

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http://dx.doi.org/10.1016/j.celrep.2013.05.011

The mechanisms by which p53 suppresses tumor growth remain ill defined. In this issue of *Cell Reports*, Timofeev et al. (2013) and Valente et al. (2013) reveal context-dependent contributions of p53-dependent apoptosis to its tumor-suppressive function.

The fact that p53 suppresses tumor growth is well established, but the mechanism by which it does so is still up in the air. After three decades of research, only this much is certain: p53 stops cancers by binding to DNA and activating transcription. The requirement for its DNA binding activity is amply demonstrated by the fact that nearly all mutations found in cancers cluster within its DNA binding domain. Additionally, the requirement for at least one of its two N-terminal transactivation domains (TADs) was elegantly demonstrated by mouse knockin experiments (Brady et al., 2011). However, a key question in the field remains unanswered: Which p53 target genes mediate its tumor-suppressive function? In this issue of *Cell Reports,* two papers, from the Strasser and Stiewe teams respectively (Valente et al., 2013; Timofeev et al., 2013), bring us closer to the answer, which is not a simple one.

At first glance, the titles of the two papers seem to state opposite conclusions regarding the role of apoptosis in p53-mediated tumor suppression, but a second look reveals little conflict, because p53 probably employs context-dependent mechanisms to prevent tumorigenesis. Upon activation, p53 drives transcription of genes acting in diverse cellular processes including apoptosis, cell-cycle arrest, senescence, DNA repair, autophagy, angiogenesis, glucose metabolism, and control of reactive oxygen species (Riley et al., 2008). Early work pointed to apoptosis, cell-cycle arrest, and senescence as the key p53 effector pathways delivering tumor suppression. However, more recent work demonstrated that p53 mutants unable to transactivate key genes in these pathways retained tumor suppression in mice (Brady et al., 2011; Li et al., 2012).

The controversy is most evident around the role of apoptosis. p53 is a direct transactivator of many genes acting in the intrinsic and extrinsic branches of the apoptotic machinery, including the pore protein BAX, the BH3-only proteins PUMA and NOXA, and the death receptor DR5 (Riley et al., 2008). PUMA is an inhibitor of BCL2-like survival factors that is required for p53-dependent apoptosis in response to diverse stimuli. In mouse models of MYC-driven B cell lymphomas, BCL2 overexpression bypasses the requirement for p53 inactivation during lymphomagenesis (Schmitt et al., 2002), and deletion of DR5, BAX, PUMA, or NOXA accelerates disease progression (Eischen et al., 2001; Finnberg et al., 2008; Michalak et al., 2009). Collectively, these data support the conclusion that apoptosis is a primary effector of p53-dependent tumor suppression. Or is it?

$p53^{-/-}$ mice are cancer prone, with >90% of animals dying of T cell lymphomas. Using knockin strategies, the Attardi and Gu teams recently generated two distinct p53 mutants, each defective in transactivation of *Puma*, *Noxa*, and the cell-cycle inhibitor *p21* (*Cdkn1a*) (Brady et al., 2011; Li et al., 2012). They demonstrated that these p53 mutant mice remain protected from thymic lymphoma, thus concluding that increased expression of these genes is dispensable for suppression of this malignancy. However, it remained formally possible that these three key p53 target genes contributed to tumor suppression when induced at low levels or when activated by other factors. To address this issue, the Strasser group generated the triple-knockout mouse $p21^{-/-}Puma^{-/-}Noxa^{-/-}$ (Valente et al., 2013). Cells from these animals showed defects in DNA-damage-induced cell-cycle arrest and senescence, and their thymocytes failed to undergo apoptosis upon DNA damage. Strikingly, none of the triple-knockout mice showed spontaneous tumor formation up to 500 days, whereas the entire $p53^{-/-}$ cohort succumbed before 250 days. So what is p53 doing to protect these mice from thymic lymphomas? The authors note that the kinetics of DNA repair upon acute DNA damage was significantly delayed in $p53^{-/-}$ dermal fibroblasts relative to wild-type cells; however, there was no delay in the triple-knockout cohorts. Furthermore, they observed that several p53 target genes involved in DNA repair were normally induced in the triple-knockout animals and suggested that the ability of p53 to orchestrate DNA repair may protect these animals from tumor development. Overall, the Strasser team reinforces the conclusion of the Attardi and Gu groups that apoptosis and cell-cycle arrest are dispensable modules of the p53 network during tumor suppression of spontaneous thymic lymphoma.

Previous work from the Stiewe team demonstrated that p53 mutants that fail to bind DNA in a cooperative fashion cannot transactivate apoptotic genes in human cells, including *PUMA* and *NOXA*, while retaining their ability to transactivate *p21* (Schlereth et al., 2010). In

order to test the role of cooperative DNA binding in vivo, the Stiewe group created mouse strains expressing "cooperativity mutant" p53 alleles ($p53^{RR/RR}$) (Timofeev et al., 2013). As expected, $p53^{RR/RR}$ cells fail to transactivate *Puma* and *Noxa* and show impaired apoptosis upon DNA damage and MYC overexpression. Interestingly, $p53^{RR/RR}$ mice are tumor prone and develop hematological malignancies, angiosarcomas, and carcinomas, suggesting that apoptosis is important for tumor suppression in this context. However, given that neither $Puma^{-/-}Noxa^{-/-}$ double-knockout (Michalak et al., 2008) nor $p21^{-/-}Puma^{-/-}Noxa^{-/-}$ triple-knockout animals (Valente et al., 2013) show such tumor propensity, we must conclude that the $p53^{RR/RR}$ phenotype is due to defects in p53 target gene transactivation beyond that of *Puma* and *Noxa*, perhaps in other branches of the apoptotic pathway. Importantly, $p53^{RR/RR}$ mice live longer than $p53^{-/-}$ mice and show fewer and more delayed appearance of thymic lymphomas. Thus, in agreement with the work from the Attardi, Gu, and Strasser teams, the Stiewe group concludes that even in the absence of apoptosis p53 can maintain some barrier against T cell lymphomas (Timofeev et al., 2013). However, in contrast to Strasser, the Stiewe group proposes that $p53^{RR/RR}$ mutants suppress these tumors via p53 target genes that mediate antioxidant functions and inhibit glycolysis, thus minimizing oxidative DNA damage, rather than through maintenance of DNA repair.

The detailed analysis of $p53^{RR/RR}$ mice reinforced the importance of context with regards to p53 apoptotic functions. In addition to the increased incidence of diverse spontaneous tumors in $p53^{RR/RR}$ mice, Stiewe and colleagues observed that $p53^{RR/RR}$ mutants could not suppress the growth of Ras/E1A-driven fibrosarcoma xenografts or MYC-driven B cell lymphomas and concluded that p53-dependent apoptosis remains an obstacle for cancer progression in the context of oncogene-driven tumorigenesis (Timofeev et al., 2013).

The current status of the field indicates that p53 employs context-dependent mechanisms of tumor suppression, with varying roles for the apoptotic program. What do these new studies suggest about p53 function in human tumors? p53 mutants that fail to effectively induce apoptosis clearly retain other activities that keep unchallenged mice free of thymic lymphomas. But humans have a much longer lifespan and undergo more environmental insults than a laboratory mouse, increasing the chances of acquiring an oncogenic driver mutation. Furthermore, thymic lymphomas are rare in the human population, and >85% of cancer deaths are caused by carcinomas, in which p53 mutations usually occur late during tumor development, after oncogene hyperactivation. As the field puts emphasis on novel p53 target genes and pathways outside of apoptosis and arrest, we must remain mindful that context is the hallmark of biological processes, and that different branches of the p53 network will contribute differentially to its tumor-suppressive function across diverse tumor types.

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